LPA2 (EDG4) mediates Rho-dependent chemotaxis with lower efficacy than LPA1 (EDG2) in breast carcinoma cells

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LPA2 (EDG4) mediates Rho-dependent chemotaxis with lower efficacy than LPA1 (EDG2) in breast carcinoma cells. Am J Physiol Cell Physiol 292: C1927–C1933, 2007; doi:10.1152/ajpcell.00400.2006.—Lysosphatidic acid (LPA) acts via binding to specific G protein-coupled receptors and has been implicated in the biology of breast cancer. Here, we characterize LPA receptor expression patterns in common established breast cancer cell lines and their contribution to breast cancer cell motility. By measuring expression of the LPA receptors LPA1, LPA2, and LPA3 with real-time quantitative PCR, we show that the breast cancer cell lines tested can be clustered into three main groups: cells that predominantly express LPA1 (BT-549, Hs578T, MDA-MB-157, MDA-MB-231, and T47D), cells that predominantly express LPA2 (BT-20, MCF-7, MDA-MB-453, and MDA-MB-468), and a third group that shows comparable expression level of these two receptors (MDA-MB-175 and MDA-MB-435). LPA3 expression was detected primarily in MDA-MB-157 cells. Using a Transwell chemotaxis assay to monitor dose response, we find that cells predominantly expressing LPA1 have a peak migration rate at 10 nM LPA that drops off dramatically at 1 μM LPA, whereas cells predominantly expressing LPA2 show the peak migration rate at 1 μM LPA, which remains high at 10 μM. Using BT-20 cells, LPA2-specific small interfering RNA, and C3 exotransferase, we demonstrate that LPA2 can mediate LPA-stimulated cell migration and activation of the small GTPase RhoA. Using LPA2 small interfering RNA, exogenous expression of LPA1, and treatment with Ki16425 LPA receptor antagonist in the BT-20 cells, we further find that LPA1 and LPA2 cooperate to promote LPA-stimulated chemotaxis. In summary, we conclude that the expression of both LPA1 and LPA2 may contribute to chemotaxis and may permit cells to respond optimally to a wider range of LPA concentrations, thus revealing a new aspect of LPA signaling.

G protein-coupled receptor; lysosphatidic acid; chemotactic migration; GTPase

LYSOPHOSPHATIDIC ACID (LPA) is a small, bioactive phospholipid that mediates multiple cellular responses, including proliferation, differentiation, motility, and survival in both normal physiology and disease. Importantly, LPA can mediate many of the “hallmarks of cancer” (9), including angiogenesis, self-sufficiency in growth signals, and tissue invasion (5, 12). These biological actions, coupled with aberrant receptor expression and elevated production of LPA in malignancies, indicate that LPA is intimately involved in tumor progression (12). In breast cancer, the role of LPA in tumor progression is highlighted by the association of autotaxin, a protein that generates LPA through its lysophospholipase D activity, with an invasive and metastatic phenotype (26). Despite the critical role that LPA plays in tumor progression, much remains to be understood about the characteristics of the receptors that mediate cellular signals from LPA.

The extracellular actions of LPA are mediated primarily through its interaction with three G protein-coupled transmembrane receptors of the endothelial differentiation gene (EDG) family, namely, LPA1 (EDG2), LPA2 (EDG4), and LPA3 (EDG7). These three receptors share a 50% sequence similarity and are known to signal through at least three families of heterotrimeric G proteins, including Giαo, Giα11, and Giα12/13. Signaling through each of these classes of G proteins mediates the downstream signaling to the Ras-MAPK, PLC-inositol 1,4,5-trisphosphate-calcium, and the Rho-Rho-associated kinase pathways, respectively. Despite their similarities in signaling, it is unclear why this redundancy in receptor expression exists if each receptor can signal similarly. Comparative studies to date have yet to uncover biochemical differences between the signaling properties of individual LPA receptors, although LPA1 and LPA2 knockout mice show different phenotypes (4, 6).

LPA1 is widely expressed in normal tissue during growth and development, whereas LPA2 expression and LPA3 expression are more restricted (5, 12). In the context of cancer, several studies have suggested that LPA1 expression in tumors is often similar to that shown in normal tissue, whereas LPA2 is associated with a more malignant phenotype in thyroid cancer (18) and carcinomas of the breast (11), ovary (7), colon (21), and stomach (25). These studies suggest that enhanced LPA2 receptor expression is important for malignant progression in cancer biology. LPA2 could contribute to tumor progression in many ways, including promoting cell proliferation and gene transcription. The latest stages of malignant progression are marked by the acquisition of an invasive phenotype. LPA contributes to tumor cell invasion through the upregulation of urokinase-type plasminogen activator and stimulation of cell motility (12). How LPA2 might contribute to this aspect of late-stage cancer is not clear, since it is LPA1, not LPA2, that is most solidly associated with cell motility (8, 12, 19). We find that BT-20 breast carcinoma cells express only LPA2, yet migrate effectively toward LPA. Therefore, we sought to determine how expression of and signaling from specific LPA receptors associate with the chemotactic motility of breast carcinoma cells.

MATERIALS AND METHODS

Cell lines and treatments. Established human breast cancer cell lines were obtained from American Type Culture Collection, except for MDA-MB-468 and BT-20 cells, which were obtained from Janet

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Price (MD Anderson, University of Texas, Houston, TX). MCF-7, T47D, BT-20, MDA-MB-157, BT-549, MDA-MB-435, MDA-MB-231, and Hs578T cells were cultured in media as recommended by American Type Culture Collection; MDA-MB-453 and MDA-MB-175 cells were cultured in low-glucose DMEM. MDA-MB-468 cells were grown in 1:1 Ham’s F12 and DMEM. All cells were cultured with 10% Fetal Pex serum (Gemini Bio-Products), 1% penicillin-streptomycin, and 1% L-glutamine. For experiments, cells (70% confluent) were trypsinized and then rinsed three times with medium plus 250 μg/ml BSA. For C3 exotransferase treatment, BT-20 cells (3 × 10^6) were electroporated with 5 μg of glutathione-S-transferase (GST) or GST-C3 exotransferase-purified protein (expression vectors provided by Dr. Keith Burridge, University of North Carolina, Chapel Hill, NC) and electroporated at 450 V and 25 μF, as described previously (23). Cells were then incubated with 6 μg/ml polymyxin B nonapeptide hydrochloride for 15 min, rinsed, plated in 10-cm dishes overnight, and then harvested for migration or RhoA activity assays.

Quantitative real-time PCR. Total RNA was extracted from cell lines using RNAqueous (Ambion). With the use of the SuperScript first-strand synthesis system (Invitrogen), cDNA was prepared from 1 μg of RNA, and one-quarter of the reaction was then used for quantitative real-time PCR (qPCR). Expression of LPA1, LPA2, and LPA3 was assessed with available probes, reagents, and the ABI7000 sequence detector as recommended by the manufacturer (Applied Biosystems).

Chemotaxis assay. Transwell chambers (6.5 mm-diameter, 8-μm pore size; Costar) were coated on both sides with 15 μg/ml collagen I for 30 min and rinsed with medium plus 250 μg/ml BSA. To assess chemotactic migration, indicated concentrations of LPA (oleoyl-L-α-LPA, 18:1; Sigma) were added to the bottom chamber. Cells (5 × 10^4) were loaded in the top of each chamber. MDA-MB-453, Hs578T, MDA-MB-157, MDA-MB-435, MDA-MB-231, MDA-MB-175, MDA-MB-453, MDA-MB-468, and BT-20 cells were allowed to migrate for 4 h at 37°C; T47D and MCF-7 cells were allowed to migrate for 22 h. Nonmigrating cells in the top chamber were removed with a cotton swab, and cells in the bottom chambers were fixed with methanol and stained with 1% crystal violet. Cells were quantitated by visual counting the cells within four separate fields (using a ×20 objective) and then taking the mean of these four fields. Cells were then incubated with 6 μg/ml polymyxin B nonapeptide hydrochloride for 15 min, rinsed, and plated in 10-cm dishes with growth medium containing 5 mM sodium butyrate. The following day, cells were rinsed with PBS and fresh growth medium was added as described previously (14). Cells were harvested 48 h after electroporation to assay for receptor expression, LPA-stimulated chemotaxis, and RhoA activity. Alternatively, cells were electroporated with control vector or LPA1 cDNA alone, and then 48 h later they were harvested for chemotaxis assays. As noted, cells were treated with 1 μM Ki16425 (Sigma) for 30 min before addition to the chemotaxis assay.

RhoA activity assay. RhoA activity was assessed with the Rhotein binding assay as described previously (15). Briefly, BT-20 cells (0.6 × 10^9) were plated onto 60-mm dishes coated with collagen I (50 μg/ml) and then treated with or without LPA (100 nM or 1 μM) as indicated for 5 min before cell extraction. Cleared extracts were incubated for 30 min at 4°C with glutathione beads (Pharmacia Biotech) coupled with GST-RBD (Rho-binding domain of Rhotein) fusion protein at 4°C and then rinsed. RhoA content of bead eluents and lysate controls were determined by immunoblotting samples using rabbit anti-RhoA antibody (Santa Cruz).

RESULTS

Expression of LPA receptors in breast cancer cell lines. Four cell surface LPA receptors have been identified in mammalian cells. Among these receptors, LPA1, LPA2, and LPA3 are the best characterized and have high affinity for LPA (5, 12). To characterize the expression patterns of LPA receptors in breast cancer cell lines, qPCR was performed to test LPA receptor mRNA expression level in individual cell lines. Values were then normalized to 18S content and reported as a relative value, arbitrarily using MDA-MB-435 LPA1 expression as 1. As shown in Fig. 1 and Table 1, the 11 cell lines tested here can be grouped into three categories according to the relative LPA receptor expression level: cells that predominantly express LPA1 (BT-549, Hs578T, MDA-MB-157, MDA-MB-231, and T47D), cells that predominantly express LPA2 (BT-20, MCF-7, MDA-MB-453, and MDA-MB-468), and a third group that shows comparable expression levels of these two receptors (MDA-MB-175 and MDA-MB-435), LPA3 was appreciably expressed only in MDA-MB-157. In general, LPA1 mRNA is expressed at much higher levels than LPA2 in the breast cancer cell lines tested here.

Breast cancer cells migrate toward LPA in different dose-response manner. To determine how LPA receptor isoform expression affects LPA-stimulated chemotaxis, we performed Transwell migration assays using collagen I, the main extracellular matrix of the stroma, as a substratum and varying concentrations of LPA as a chemoattractant. With this assay, we tested the dose response of Hs578T cells, which predominantly express LPA1, and BT-20 cells, which express only...
Table 1. Comparative expression of LPA receptors in commonly used breast carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>EDG2 (LPA1)</th>
<th>EDG4 (LPA2)</th>
<th>EDG7 (LPA3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-435</td>
<td>1.00</td>
<td>0.88</td>
<td>0.00045</td>
</tr>
<tr>
<td>T47D</td>
<td>5.76</td>
<td>0.66</td>
<td>0.00047</td>
</tr>
<tr>
<td>Hs578T</td>
<td>10.30</td>
<td>0.096</td>
<td>0.009</td>
</tr>
<tr>
<td>MDA-MB-157</td>
<td>11.13</td>
<td>1.59</td>
<td>2.75</td>
</tr>
<tr>
<td>BT-549</td>
<td>29.37</td>
<td>0.55</td>
<td>0.17</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>43.32</td>
<td>0.81</td>
<td>0.0045</td>
</tr>
<tr>
<td>MDA-MB-175</td>
<td>3.24</td>
<td>4.93</td>
<td>0.0082</td>
</tr>
<tr>
<td>MCF7</td>
<td>0.10</td>
<td>4.97</td>
<td>0.00084</td>
</tr>
<tr>
<td>MDA-MB-453</td>
<td>0.25</td>
<td>2.24</td>
<td>0.025</td>
</tr>
<tr>
<td>BT-20</td>
<td>0.0011</td>
<td>3.09</td>
<td>Not detectable</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>0.13</td>
<td>5.48</td>
<td>0.34</td>
</tr>
</tbody>
</table>

LPA, lysophosphatidic acid. All values are reported as level of message normalized to 18S ribosomal RNA as quantified by real-time PCR and relative to MDA-MB-435 LPA1 values.

LPA2. Interestingly, these two cell lines displayed very different dose responses toward increasing concentrations of LPA. The Hs578T cells display migration toward 10 nM LPA, which is significantly increased by 100 nM LPA. When the concentration of LPA is increased to 1 or 10 μM, chemotactic migration of the Hs578T cells is reduced to the basal level (Fig. 2A). The BT-20 cells, in contrast, show an increasing rate of migration with higher concentrations of LPA, which peaks at 1 μM and remains high at 10 μM (Fig. 2B). To determine the generality of this trend in dose response, other breast carcinoma cells that primarily express LPA1 or LPA2 were assessed for chemotactic migration at 100 nM or 1 μM LPA and compared with unstimulated (basal) response. As seen in Fig. 2, C–E, breast carcinoma cell lines expressing predominantly LPA1 (MDA-MB-231, T47D, and MDA-MB-157) display the highest migration rate at 100 nM, whereas migration rates at 1 μM are frequently reduced to the basal level. Interestingly, MDA-MB-231 cells, which have very high expression of LPA1, show very high spontaneous migration without LPA and maintain a high rate of migration at 1 μM LPA. BT-549 and MDA-MB-175 cells, in contrast, do not migrate toward LPA (data not shown). In the LPA2 predominantly expressing group, MDA-MB-468 cells show the same pattern as BT-20 cells; however, LPA inhibits MDA-MB-453 and MCF7 cell migration (Fig. 2, F–H). The results suggest that both LPA1 and LPA2 can act as conditional migratory receptors for breast cancer cells such that the expression of the receptor does not always confer the ability of cells to chemotax toward LPA. Notably, LPA2-expressing cells migrate toward higher concentrations of LPA than those that express only LPA1.

LPA2 mediates migration through RhoA activation in BT-20 cells. Previous studies have shown that LPA2 is overexpressed in several types of cancer (7, 11, 21). Although most studies report LPA1 as the primary LPA receptor involved in cell motility, different mechanisms underlying LPA2-mediated migration have been reported (20, 24). The small GTPase RhoA is a well-accepted mediator of LPA signaling, studied mainly in several types of cancer (7, 11, 21). Although most studies show that LPA2 is overexpressed in BT-20 cells with siRNA specific to LPA2 and then measured chemotactic migration (Fig. 3C) or RhoA activation (Fig. 3E) in response to stimulation with 1 μM LPA. The results show that migration was substantially decreased in cells treated with siRNA specific to LPA2 compared with the untreated and nontargeted siRNA-treated cells (Fig. 3C). In Fig. 3D, qPCR confirmed that LPA2 expression was reduced by ~80% by the LPA2 siRNA compared with the untreated or the nontargeted control. The results demonstrated that LPA2 is the major receptor that mediates LPA-stimulated migration in BT-20 cells.

To conclusively determine that LPA2 and not another unidentified LPA receptor mediates this migration and RhoA activation, we treated BT-20 cells with siRNA specific to LPA2 and then measured chemotactic migration (Fig. 3A, C) or RhoA activation (Fig. 3E) in response to stimulation with 1 μM LPA. The results show that migration was substantially decreased in cells treated with siRNA specific to LPA2 compared with the untreated and nontargeted siRNA-treated cells (Fig. 3C). In Fig. 3D, qPCR confirmed that LPA2 expression was reduced by ~80% by the LPA2 siRNA compared with the untreated or the nontargeted control. The results demonstrated that LPA2 is the major receptor that mediates LPA-stimulated migration in BT-20 cells.

Next, we assessed the role of LPA2 on RhoA activation stimulated by LPA. As shown in Fig. 3E, 1 μM LPA treatment leads to a robust activation of RhoA both in untreated or nontargeting siRNA-treated cells. However, reducing LPA2 expression by specific siRNA treatment abolishes RhoA activation. Together, these observations indicate that LPA2 mediates the activation of RhoA, which is required for the chemotactic migration of BT-20 cells toward LPA.

LPA1 and LPA2 cooperate to enable cells to respond to a wider range of LPA concentrations. Cells expressing either LPA1 or LPA2 display different migratory dose responses toward LPA. Therefore, we hypothesize that expression of both LPA1 and LPA2 would increase the migratory potential by facilitating cell migration over a wider range of LPA concentrations. To test this hypothesis, LPA2 siRNA and a LPA1 cDNA construct were introduced into BT-20 cells by electroporation using nontargeting siRNA and pcDNA3 as respective controls. Cells were then assessed for chemotactic migration toward 100 nM and 1 μM LPA, which represent the concentrations for peak chemotactic responses for LPA1 and LPA2, respectively. Here, we find that depletion of LPA2 by siRNA effectively decreased cell migration toward LPA at 100 nM and 1 μM LPA. Exogenous expression of LPA1 increased the basal rate of migration (Fig. 4A) and enhanced cell migration to 100 nM LPA (Fig. 4A) in nontargeting and LPA2 siRNA-treated cells. However, LPA1 expression did not affect cell migration at 1 μM LPA (Fig. 4A). Figure 4A, inset, depicts the specific contributions of LPA1 expression. Notably, there is no difference in LPA1 contributions to cell motility in the presence or absence of LPA2, suggesting that LPA1 and LPA2 can cooperate additively. Figure 4, B and C, show the efficiency of...
LPA2 siRNA and LPA1 exogenous expression in the cells, respectively.

To confirm these observations, we utilized the LPA receptor antagonist Ki16425, which selectively antagonizes LPA1 and LPA3 (17) (Fig. 5). For these experiments, BT-20 cells were transiently transfected with either pcDNA3 or a cDNA for LPA1. After 48 h, cells were suspended and then left untreated or treated for 30 min with 1 μM Ki16425. Cells were then assessed for chemotactic migration toward the indicated concentration of LPA (18:1; Sigma) using collagen I-coated Transwell chambers for 4 h (A–C and E–G) or 22 h (D and H). Bars represent SD of the mean number of cells migrated per field from triplicate determinations. Representative assays from at least 3 separate experiments for each cell line are shown.

DISCUSSION

In the present study, we uncover several important properties of LPA receptor signaling. By initially investigating the expression of LPA receptors on various breast carcinoma cell lines, we determine that LPA2 can function as a conditional migratory receptor in breast cancer cells. We further find that
LPA2 can promote a robust activation of RhoA and that cell migration mediated by LPA2 requires functional Rho small GTPases. In addition, we determine that LPA1 and LPA2 differ in their dose response to LPA, in which LPA1 promotes migration at lower concentrations of LPA. Finally, we show that LPA1 and LPA2 can cooperate to promote motility of breast cancer cells over a wider range of LPA concentrations, which is likely to have important biological implications.

One of the principal findings of our present study is a previously unrecognized difference in the efficacy of signaling between LPA1 and LPA2. We find that LPA1 optimally mediates chemotactic signaling at lower concentrations of LPA than does LPA2. This finding is surprising for several reasons. First, LPA1 and LPA2 have been suggested to signal through similar pathways involving Gi, Gq, and G12/13 family heterotrimeric G proteins, Rac, Rho, Ras, and MAPK (5, 12). Also, although LPA1 and LPA2 are both classified as high-affinity LPA receptors, LPA2 is reported to have a higher affinity for LPA than LPA1. The EC50 of LPA2/EDG4 for 1-oleoyl LPA, the LPA species used here, is actually reported to be substantially higher than that for LPA1/EDG2 (2). These observations clearly emphasize that there exists a different efficiency in transducing signals that mediate chemotactic migration between the two EDG family receptors. One possible explanation for this difference may lie in the receptor inactivation by endocytosis. The bell-shaped response curve seen with LPA1-mediated migration is commonly observed with receptors that undergo endocytic downregulation and degradation. At high concentrations of LPA (10 μM), LPA1 is known to be rapidly endocytosed (12, 13). The exception to this phenomenon is seen with the MDA-MB-231 cells, which are able to maintain a high rate of migration at 1 μM LPA. Notably, these cells express very high levels of LPA1, which could saturate the endocytic mechanisms and effectively slow the rate of endocytosis, thus slowing the downregulation of the receptor, as seen with EGF receptor overexpression (1).
though data on ligand-mediated endocytosis and trafficking of LPA2 have not been reported, the progressive increase in cell motility at high levels of LPA suggests that LPA2 may not undergo endocytic trafficking in the same manner as LPA1 in response to ligand. Certainly, further comparative studies are needed to elucidate the mechanistic basis that defines the difference in efficacy of signaling from these receptors.

It has been suggested that, because multiple LPA receptors of the EDG family can be expressed on individual cells, they are likely to signal in a cooperative manner; however, this concept remains to be tested (12). Our results suggest that distinct LPA receptors can, in fact, work together to facilitate chemotactic migration. This cooperation is manifested in two ways. First, LPA1 and LPA2 work additively, rather than competitively, when assessed at a LPA concentration that both receptors can respond, namely 100 nM LPA. It is easy to envision competition between the two receptors for downstream signaling intermediates. It is well documented, for example, that heterotrimeric G proteins are often a limiting factor in G-protein-coupled receptor signaling. If the two receptors competed for signaling intermediates such as G proteins, LPA1 expression in the BT-20 cells would not have promoted cell migration to the same degree with or without LPA2 siRNA treatment (depicted in Fig. 4A, inset). Therefore, these data suggest that the two receptors can cooperate in this manner. In the second mode of cooperation, signaling through LPA1 and LPA2 permits cells to respond to a broader range of LPA concentrations than a single receptor alone could achieve. We find that LPA1 is more efficacious than LPA2 at promoting cell motility at lower concentrations of LPA such as at 10 and 100 nM. LPA2, operating predominantly at high levels of LPA, could cooperate with LPA1 to extend the range of LPA concentrations to which a cell could respond.

The cooperation between LPA1 and LPA2 has important biological implications. In various cancers, LPA is known to accumulate in tissues and body effluents such as plasma and ascites, which in turn is associated with a poor prognosis (12). For example, LPA in the ascites of ovarian cancer patients is reported to range from 1 to 80 μM (12). This level of bioaccumulation of LPA would be expected to effectively shut down LPA1 due to its high rate of ligand-dependent endocytosis, whereas LPA2 could be expected to take over as the dominant signaling receptor. Other studies on LPA receptors, although not comparing LPA1 and LPA2 directly, have shown the same dose-response curve for proliferation for cells that primarily express either LPA1 or LPA2 as we observed for cell motility (3, 19, 20). Therefore, the observation that LPA2 mediates both proliferative and chemotactic signals at high LPA concentrations may, in fact, be studying the reduced capacity of LPA1 to signal or the effects of low levels of other LPA concentrations. These results may help to explain why LPA2 is associated with tumor progression in tumor types in which LPA production is known to be high.

Our study highlights what happens when LPA2 functions as a migratory receptor. Although LPA2 can promote cell migration, we find that the expression of the LPA2 receptor does not necessarily confer the ability to migrate toward LPA. That is, LPA2 is a conditional migratory receptor. The observations that cells expressing LPA2 were not migratory, even though LPA2 continues to function for cell proliferation, led to the initial conclusion that LPA1 was the principal LPA receptor for cell migration (12, 19, 22). Subsequent studies have noted that LPA2 acts as a migratory receptor only under distinct conditions. One such study indicated that the focal adhesion-associated protein TRIP6/ZRP-1 is a required cofactor for LPA2 to engage the cell migration machinery by recruiting LPA2 to focal adhesions and the actin cytoskeleton (24). Another study suggested that LPA2 cooperates with c-Met, the receptor for hepatocyte growth factor, to facilitate migration. They find that stimulation with low concentration of hepatocyte growth factor revealed the ability of LPA2 to facilitate migration through the transactivation of c-Met (20). Here, we find that two of the four breast cell lines that have substantial levels of LPA2 message are able to migrate toward LPA. Interestingly, these two migratory cell lines have c-Met, whereas the two cell lines that are unable to chemotax toward LPA do not (data not shown). However, effective reduction of c-Met levels by siRNA does not affect LPA2-mediated chemotaxis in these cells (unpublished observation). Clearly, the mechanisms governing this conditional migratory phenotype are complex and will require further study.

The results presented here have important implications for the interpretation of the current literature on LPA signaling. Commonly used concentrations of LPA vary widely from 10 nM to 10 μM and higher, where 10 μM LPA is the more common concentration for studying the effects of LPA on signaling and biological function. If our observations that LPA1 functions are shut down at high levels of LPA (1 μM and higher) in most breast carcinoma cells extend to other cell types, studies reporting the function of LPA1 at high concentrations may, in fact, be studying the reduced capacity of LPA1 to signal or the effects of low levels of other LPA receptors. Therefore, comparison of results from various studies without taking into consideration what concentration of LPA was used could lead to an erroneous conclusion, potentially connecting LPA1 signaling properties to LPA2 or vice versa.

Although many questions remain to be answered regarding their biochemical properties and how the individual LPA receptors signal, our present study helps to increase our under-
standing on the functional differences between LPA receptors. Although we cannot comment on the effect of overall protein levels of LPA1 and LPA2 to affect LPA signaling based on the results of this study, we can make conclusions based on the receptor type expressed and how receptor isoform expression may affect cell motility in response to the concentration of LPA. We find that cell lines expressing LPA1 that are able to migrate show a peak migration at 100 nM LPA, whereas the migration of LPA2-expressing cell lines peaks at 1 μM regardless of the expression of the receptors on the mRNA level. Therefore, we suggest that the expression of distinct LPA receptor expression affects the dose response rather than the amplitude of the response to LPA. In summary, we find that LPA2 can effectively mediate the activation of RhoA and Rho-mediated migration of breast cancer cells. Furthermore, LPA2 promotes chemotactic migration toward higher concentrations of LPA, thus suggesting that LPA2 is less efficacious than LPA1. Finally, we conclude that LPA1 and LPA2 can cooperate to promote efficient chemotactic migration over a wider range of LPA concentrations than either receptor can mediate alone.

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